ORIGINAL ARTICLE

Green Robusta Coffee Bean Extract (GRCBE) Inhibits Nickel and Cobalt-Induced Radical Superoxide Production in Human Peripheral Blood Mononuclear Cells

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ABSTRACT

Introduction: Nickel (Ni^{2+}) and cobalt (Co2+) are notorious to induce apoptosis mediated by reactive oxygen species (ROS). Several factors such as usage period, acidity level (pH), and food consumed can increase the release of metal ions, particularly Ni^{2+} and Co^{2+} ions. The released Ni2+ ions can bind to TLR4 receptors and therefore stimulate the proliferation and activation of PBMCs. Green Robusta green coffee extract (GRCBE) is expected to inhibit cell apoptosis and cell proliferation because of its chlorogenic acid, polyphenol. It is a promising candidate as an effective antioxidant. This study aimed to investigate the potential effect of GRCBE against radical superoxide in PBMC cells exposed to Ni^{2+} and Co^{2+}. **Materials and Methods:** The metal included $NiCl_2.6H_2O$ and $CoCl_2.6H_2O$. The coffee extract was derived from East Java Robusta coffee beans. The beans underwent maceration in 97% ethanol and were subsequently diluted into three concentrations: 31.25, 62.5 and 125 µg/ml. The NBT assay was employed to assess the production of ROS and quantify the level of oxidative stress. **Results:** The GRCBE at a dose of 125 µg/ml showed the most effective reduction in radical superoxide in PBMC cells triggered by Ni^{2+} and Co^{2+}. Lower concentrations of 62.5 µg/ml and 31.25 µg/ml also displayed some reduction. **Conclusions:** The presence of Ni^{2+} and Co^{2+} has a significant oxidizing effect on PBMC, but GRCBE can inhibit the formation of superoxide radicals.
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INTRODUCTION

Metals used in dentistry are never used as a single metal. It is alloyed with other metals to achieve the best physical, mechanical, biological, chemical, and aesthetic properties. Nickel (Ni^{2+}) and cobalt ions (Co^{2+}) are usually combined with chromium (Cr). These alloys are still widely used in metal-ceramic crowns and partial denture frames (1). Alloys interact with the wet environment in the oral cavity. Several variables can influence the release of ions, including the oral environment influenced by parameters like temperature, pH, saliva, external substances like tobacco and alcohol, immunological responses, coexistence of various metals, and the structure and surface treatment of dental alloys (2, 3). Metal ions released can contact and react with nearby cells and tissues, such as the oral epithelium and mucosa, and therefore can be distributed throughout the body (4).

Ni^{2+} and Co^{2+} are the most reactive, cytotoxic, and can induce cell death. They can interact with molecular oxygen to form superoxide radicals or superoxide (O_2) anions. Superoxide radicals are a type of ROS (3, 5). It is a free radical species capable of binding the electrons from the molecules around it; therefore, it will form a new free radical (chain reactions) (6). Oxidative stress is a pathological situation that arises from an imbalance between the generation and elimination of free radicals. Oxidative stress induces lipid peroxidation in cell membranes, DNA damage, protein damage, activation of many transcription factors associated with stress, and the generation of proinflammatory cytokines and anti-inflammatory substances. Peripheral blood mononuclear cells (PBMCs) play a crucial role in mediating the immune system and inflammatory response. PBMCs comprise lymphocytes, monocytes, natural killer cells

(NK cells), and dendritic cells (DCs) (7).

Oxidative stress can be prevented by antioxidants (7, 8). One plant that contains antioxidants is coffee beans. Bioactive components of coffee beans, such as flavonoids, xanthine alkaloids, and polyphenols, have been known to function as immunomodulators, anti-inflammatory, antibacterial, and antioxidant activities that can maintain cell life. The effectiveness of green Robusta coffee bean extract (GRCBE) in decreasing free radical superoxide can be ascribed to its abundant concentration of chlorogenic acid and other bioactive components.Green coffee beans contain a high concentration of antioxidants, which aid in the reduction of free radicals within the body (9). Chlorogenic acid possesses various health advantages, such as its ability to act as an antioxidant, reduce inflammation, and lower blood pressure (10). A comparative analysis of the antioxidant activity of extracts from green and roasted coffee beans revealed that both extracts shown robust capabilities in scavenging free radicals (11). A separate investigation evaluated the local anti-inflammatory properties of the methanol extract derived from green coffee beans and documented favorable outcomes (12). The findings indicate that GRCBE is efficacious in diminishing free radical superoxide and its related oxidative stress. It is crucial to note, however, that the efficiency of GRCBE may vary depending on parameters such as extraction method, concentration, and individual response. Additional investigation is required to gain a comprehensive understanding of the possible advantages of GRCBE in diminishing free radical superoxide and its influence on general well-being.

Unfortunately, thus far, there have been insufficient findings that explicitly address the inquiry regarding the efficacy of GRCBE in diminishing free radical superoxide in PBMC cells, specifically those that have been exposed to dental metals. Nevertheless, several studies have investigated the antioxidant capabilities of GRCBE and its potential advantages for human health. One study shown that the use of GRCBE led to a considerable decrease in the abundance of Streptococcus mutans, a bacteria known to contribute to the development of dental caries (13). A separate study demonstrated that the use of GRCBE resulted in a reduction in blood pressure in rats with spontaneous hypertension (14). Moreover, specific research studies have examined the antioxidant effectiveness of coffee extracts, particularly those obtained from unroasted coffee beans, and their ability to eradicate detrimental free radicals such as superoxide and hydroxyl radicals (15, 16). Although these studies are not directly related to this issue, they suggest that GRCBE may have some potential health benefits because of its antioxidant properties. Hence, additional research is necessary to ascertain the efficacy of GRCBE in diminishing the presence of free radical superoxide in PBMC cells that have been subjected to Ni^{2+} and Co^{2+}. In addition, optimal GRCBE concentrations that can reduce the production of superoxidase radicals due to nickel and cobalt will be acquired

MATERIALS AND METHODS

Ethical clearance and study design

This study employed a quasi-experimental laboratory setup, utilising a descriptive observational design and conducting in vitro studies. The study obtained ethical permission from the Ethical Research Committee of the Faculty of Dentistry, University of Jember, East Java, Indonesia, under appointment number 2195/UN.25.8/KEPK/DL/2023.

Metal Chemicals

The metal allergens used in the experiment were nickel (II) chloride hexahydrate ($NiCl_2.6H_2O$) and cobalt (II) chloride hexahydrate ($CoCl_2.6H_2O$) obtained from Fluka/Riedel de Ha∧n. These chemicals were dissolved in Iscove's modified Dulbecco's medium (IMDM) from Biowhittaker, Verviers, Belgium, which contained 10% heat-inactivated foetal calf serum (FCS, Hyclone, Logan USA). $NiCl_2.6H_2O$ and $CoCl_2.6H_2O$ metal solutions used a non-toxic concentration of 125 μM. TAK-242 and BAY-11-708 (Sigma Chemical Co. (St. Louis, MO, USA)) were used for the TLR-4 and NFkB blocker analysis (17-20).

Extraction of peripheral blood mononuclear cells (PBMCs)

Peripheral blood mononuclear cells (PBMCs) were obtained from 30 ml of recently collected venous blood from four distinct healthy individuals without any documented metal allergies. The inclusion criteria for donors consisted of persons who were in good health and had no prior history of metal allergies or hypersensitivity to common allergenic metals, such as nickel, cobalt, or palladium. Individuals who have not been exposed to recognised allergenic metals through profession or environmental causes, donors who do not currently have any cancerous condition, adherence to research criteria, including being within the age range of 20-50 years, abstaining from smoking, and refraining from consuming alcohol or drugs. The exclusion criteria were as follows: Individuals with a medical history of metal allergies or documented hypersensitivity to specific metals, as well as those who have been exposed to known allergenic metals in their occupation or environment, should be excluded from the study. Additionally, individuals with active cancer or a history of cancer should only be considered for inclusion on a case-by-case basis, following specific criteria. Any other health conditions or medications that may impact the immune system or compromise the reliability of the research results should also be considered.

The isolation process involved using Ficoll (Lymphoprep, Fresenius Kabi Norge AS) density gradient centrifugation.

The cells counting was performed utilising a CASY cell counter (Schᴧrfe system, TT-2-BA-1007, Rutlingen Germany) in conjunction with trypan blue. PBMCs were rinsed with IMDM culture media and seeded in 75 cm^2 culture flasks (T75, Cellstar Greiner bio-one) at a density of 50 x 106 cells per 10 ml.

Preparation of green Robusta coffee bean extract (GRCBE)

The Robusta coffee beans were extracted from the coffee and cocoa fields located in Jember, East Java, Indonesia. The Robusta Coffee bean was identified before to the study by obtaining it from The Crop Laboratory, Department of Agricultural Production Jember State Polytechnic's in Indonesia (53/PL17.3.1.02/LL/2018). The preparation of GRCBE (Coffea canephora) was performed using maceration weigh 250 g of crushed Robusta coffee bean and macerate it in 1000 ml, 96% ethanol solution for 48 h. Subsequently, the solution underwent filtration and evaporation via a rotary evaporator, maintaining a temperature range of 30-40˚C. The extract was thinned with IMDM to achieve concentrations of 31.25, 62.5, and 125 µg/ml.

GRCBE toxicity experiments

To determine suitable concentration ranges of GRCBE, the cytotoxicity was assessed using the MTT reduction assay, which involves the use of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich, Inc St Louis USA). Around 100 µl of cells (with a concentration of 5 x 104 cells per ml) were placed in 96-well culture plates and subjected to escalating doses of GRCBE. Following a 24-hour incubation period, the culture medium was extracted and 50 µl of MTT solution (5 mg/ml) was introduced into each well. The MTT solution was freshly produced and dissolved in H_2O, then filtered through a 0.22-µm filter. The plates were placed in a dark environment and kept at a temperature of 37˚C. Following an incubation period of 2-3 hours, 50 µl of DMSO (Merck, Darmstad, Germany) was introduced into each well. The solution was then shaken and subsequently analysed using an ELISA reader to detect the optical density (OD) at 570 nm (HumaReader HS, HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany). The optical density (OD) of the cells in the absence of metal was designated as 100%. The viability of exposed cells was assessed by calculating the ratio of the optical density (OD) of the experimental sample to the OD of the control cells, and then multiplying it by 100%.

TLR-4 blocker experiment

TAK 242 and BAY-11-7082, obtained from Sigma-Aldrich in St. Louis, MO, USA, were subjected to $NiCl_2$ and $CoCl_2$, in addition to the application of GRCBE. The effects of GRCBE inhibitors were simulated by decreasing ROS production in PBMCs.

Cell attachment and NBT experiments

Prepare 24-well plates and insert a sterile slipcover into each well. Seeded 100 µl/well PBMC above the prepared slipcover. Cell attachment was performed by incubating cells at a temperature of 370C for 20 min. Following incubation, a volume of 1 ml of IMDM was introduced and subjected to a subsequent incubation at a temperature of 37°C for a duration of 20-30 minutes. The samples were categorized into 8 categories according on their incubation stages of GRCBE, each lasting for 1 hour. Incubation of metal was done for 1h and NBT solution for 1.5h. Observed under an inverted microscope, shaking gently to observe cell attachment. Then washed using IMDM 3 times, carefully.

After the medium was taken and ensure PBMC cells have attached to the glass cover then washed twice using IMDM, and then fixed with methanol. Staining was performed using safranin solution. The ROS production was examined by counting the number of PBMC cells that were purplish blue of blue formazan particles. The cell percentage was determined by randomly counting 100 PBMC cells in each group using a 1000x magnification microscope lab opt from 3–5 observed fields of view by 3 examiners. PBMC cells producing free radicals were then counted and the results were averaged.

Data analysis

The statistical significance of the impact of GRCBE on the suppression of radical superoxide caused by exposures to Ni^{2+} and Co^{2+} was assessed using one-way ANOVA with GraphPad Prism Software version 10.0 (San Diego, CA, USA). A p-value ≤ 0.05 was statistically significant. The data are presented in the mean values ± standard deviation (SD).

RESULT

Viability of PBMCs after exposure to GRCBE

The possible antioxidant effects of GRCBE on PBMCs were initially investigated to determine the optimal concentrations that are both effective and non-toxic. Cytotoxicity tests were conducted utilising MTT reduction assays as a means of measurement. Initial evaluations were performed to ascertain the bioavailability of GRCBE to PBMCs, using a concentration range of 500 - 31.25 µg/ml. Following a 24-hour exposure of PBMC to escalating doses of GRCBE, it was determined that concentration ranges of 31.25 – 125 µg/ml were suitable. MTT viability analysis revealed that the control group exhibited the maximum average cell percentage of 99.5%, while the lowest viability of PBMC cells was observed at a concentration of 500 µg/ml of GRCBE, with a viability of 52.5%. The survival rate of PBMCs following exposure to various concentrations of GRCBE was as follows: 31.25 µg/ml (93.25%), 62.5 µg/ml (89%), 125 µg/ml (86.75%), and 250 µg/ml (68.25%). However, the specific data is not shown.

Analysis of the antioxidant capacity of GRCBE

The production of superoxide radicals can be observed microscopically, which is characterized by the formation of blue or purplish-blue formazan particles on PBMC cell membranes. The results of calculating the number of PBMC cells that produce superoxide radicals are shown in Fig. 1.

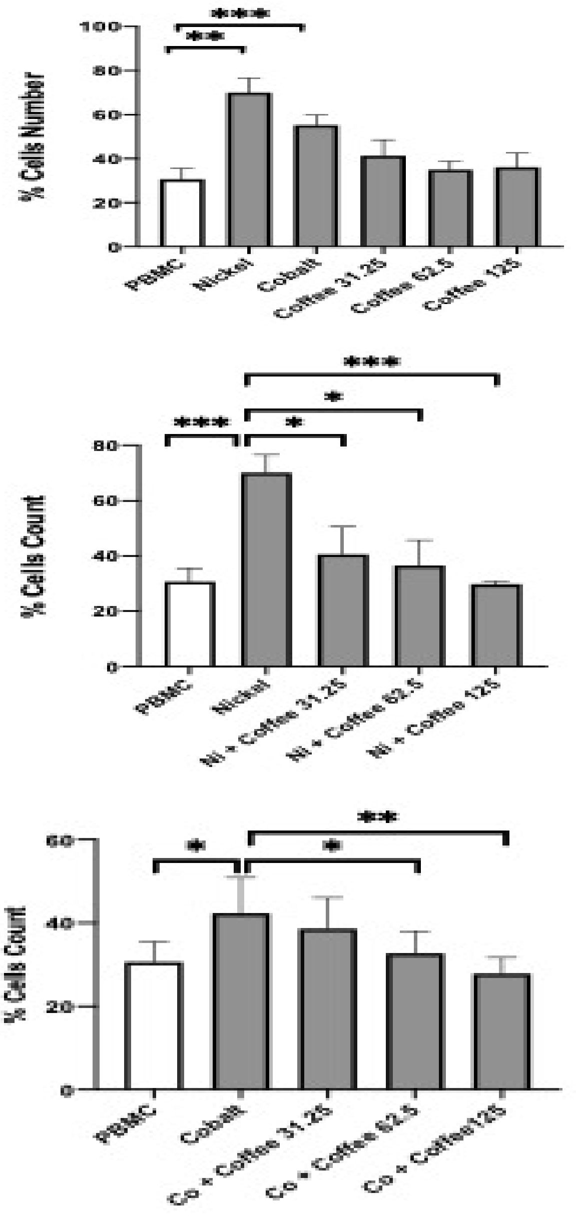
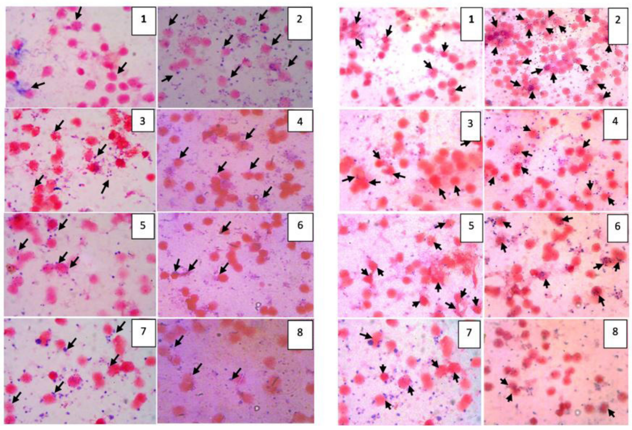


Figure 1. Bar chart of the average increase in radical superoxide in the PBMC control (A) and in the PBMC exposed to Ni (B) and Co (C) in the presence and/or absence of GRCBE. Bars represent mean ± SD from four independent experiments (PBMC n = 4 experiments) ($p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) (One way ANOVA).

Figure 1A shows a significant rise in the generation of superoxide radicals following exposure to $NiCl_2$ and $CoCl_2$, in comparison to the PBMC control group and GRCBE at doses of 31.25, 62.5, and 125 µg/ml. The $NiCl_2$ exhibited the most significant elevation in superoxide radical generation, reaching a value of 76.06%. Similarly, the CoCl2 demonstrated a substantial increase of 48.33%. In contrast, in the GRCBE-exposed group, at all concentrations did not show significant results compared with the control. The greatest elevation in superoxide radical generation (41.39%) was noted when using a concentration of 31.25 µg/ml of GRCBE (Fig. 1A). Meanwhile, Figure 1B and 1C demonstrate a decreasing tendency following exposure to GRCBE.

The group exposed to GRCBE showed the greatest rise in superoxide radical generation (41.39) at a dose of 31.25 µg/ml (Fig 1A). Meanwhile, Figures 1B and 1C exhibit a decreasing tendency. Evidently, there was a significant reduction in radical superoxide levels following the treatment of $NiCl_2$ and $CoCl_2$ with GRCBE at various doses, spanning from low to high. A significant reduction in ROS was seen when GRCBE was present at a concentration of 125 µg/ml. Asterisks (*** and/or **) signify a strong significance with a p-value ≤ 0.01.



2(a) 2(b)|

Figure 2. Represent microscopic images of PBMC cells that produce superoxide radicals. Superoxide radical production is indicated by black arrows (A) NiCl2 and (B) CoCl2, each treatment was (1) cell control, (2) metal control, (3) GRCBE 31.25, (4) GRCBE 62.5, (5) GRCBE 125 µg/ml, (6) metal + GRCBE 31.25 µg/ml, (7) metal + GRCBE 62.5 µg/ml, (8) metal + GRCBE 125 µg/ml

In the microscopic images (Fig. 2) the production of superoxide radicals is indicated by a black arrow. In the PBMC exposed $NiCl_2$ (Fig. 2A-2) and CoCl2 (Fig. 2B-2) groups, the production of superoxide was more robust than that in the PBMC control group (Fig. 2A-1 and 2B-1) and gradually reduced after the metal exposure group was treated with various concentrations of GRCBE (Fig. 2A-6-8 and 2B-6-8). In the graph, the most abundant reduction in superoxide radical production in metal

+ GRCBE 125 μg/ml. Overall microscopic images showed that in the metals exposed to three different GRCBE concentrations 31.25 μg/ml (Fig. 2A and B-6), 62.5 μg/ml (Fig. 2A and B-7), and 125 μg/ml (Fig. 2A and B-8) showed a decrease in superoxide radical production when compared with the $NiCl_2$ and $CoCl_2$ metal control group (Fig. 2A, B-2). Microscopic images of PBMC cells that produce superoxide radicals, which are characterised by blue formazan around the cell membrane, are shown in Fig. 2.

Analysis of the antioxidant capacity of the TLR-4 blocker experiment

Further experiments were conducted to investigate whether GRCBE has a strong effect in inhibiting superoxide radicals due to exposure to Ni^{2+} and Co^{2+} after GRCBE treatment compared with both chemical blockers typically used in the inhibition of TLR-4 signalling. Fig. 3 showed that there was significant inhibition of Ni^{2+} after treatment using GRCBE 125 μg/ml, which is also comparable to the results of inhibition by TAK 242 and BAY 11–7082 (Fig. 3A). In the cobalt exposure group, significant results were obtained after treatment using GRCBE 125 μg/ml, whereas those using TAK 242 and BAY 11–7082 blockers showed slight inhibition of superoxide radicals due to cobalt but statistically not significant (Fig. 3B).

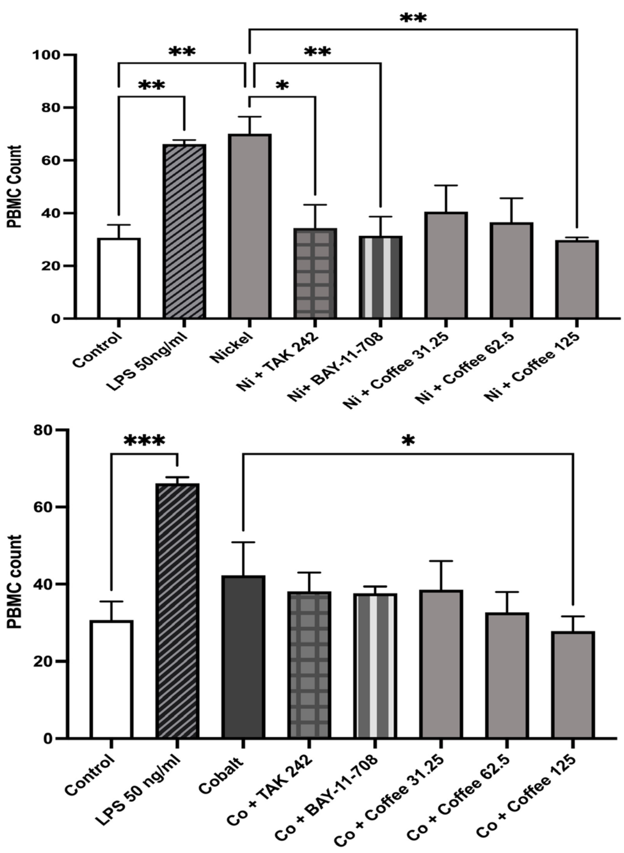


Figure 3. The average bar chart graph of PBMC cells in radical superoxide reduction in the GRCBE treatment group of (A) NiCl2 and (B) CoCl2 compared with two types of commonly used TLR-4 blockers: TAK 242 and BAY 11-7082 ($p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) (One way ANOVA)

Overall, based on the results obtained, the highest superoxide radical production in PBMC cells was found in the nickel-exposed group (76.06%) and cobalt-exposed group (48.33%), whereas the lowest superoxide radical production was found in the treatment group exposed to GRCBE concentrations of 125 μg/ml and $NiCl_2$ amounting to 29.78% and $CoCl_2$ 27.83% (Fig. 3A, 3B).

The data analysis conducted a normality assessment using the Shapiro-Wilk test and a homogeneity assessment using the Levene test. The test findings indicate that the data have a normal distribution and are homogeneous, with a p-value greater than 0.05. In addition, one-way analysis of variance (ANOVA) was performed, revealing a statistically significant difference in the results ($p < 0.05$). The LSD test generated a significance level of $p < 0.05$. Additionally, there was a group that exhibited a significance level of $p > 0.05$ but demonstrated a statistically significant decrease in superoxide radicals.

DISCUSSION

This laboratory experiment aims to assess the efficacy of GRCBE in mitigating the generation of superoxide radicals in PBMC cells when exposed to $NiCl_2$ and $CoCl_2$, which are reactive metals known to behave as pathogens (17). The presence of metals in the oral cavity can lead to corrosion by the emission of Ni2+ ions. Several variables can influence the release of metal ions into the mouth, including temperature, pH of saliva, external substances including tobacco and alcohol, food, drinks, and various metal alloys. These variables can initiate corrosion, leading to interactions between metals and adjacent cells (2). The interaction of metals with cells can lead to increased production of superoxide radicals (21). In this study, we used the metal salts $NiCl_2$ and $CoCl_2$ as materials resembling Ni^{2+} and Co^{2+} ions released in the oral cavity.

Superoxide radicals, a category of reactive oxygen species (ROS), are typically generated by the enzyme NADPH oxidase as a means of protecting against infections. Superoxide radicals are usually anion radicals that are then removed through a dismutation reaction by the SOD enzyme (22). The production of superoxide radicals can be determined using the NBT test. Superoxide radicals will later react with cytochrome C or nitrogen blue tetrazolium (NBT) to produce a blue formazan colour (23).

The results in the cell control showed that the number of PBMC cells that produced superoxide radicals was 30.67% per 100 cells. In the metal group, there was a robust increase in superoxide radical production of 76,06 % ($NiCl_2$) and 48.33% ($CoCl_2$). Free radicals due to the reaction of metal ions affect PBMC cells. PBMCs bind to Ni^{2+} and Co^{2+} ions via TLR4 receptors (17). Ni^{2+} and

Co^{2+} enter the PBMC cell via a divalent metal transporter (DMT1) (24). This agrees with several studies that have shown that dental alloys made of nickel and cobalt may increase free radical superoxide levels and cause oxidative stress (21, 25). Ni^{2+} and Co^{2+} ions can react with hydrogen peroxide through the Fenton reaction to generate hydroxyl radicals (25). The Fenton reaction is a reaction between metal ions and hydrogen peroxide to form hydroxyl radicals (H2O2 + Fe2+ →Fe3+ + • OH + -OH). Dental or orthodontic alloys increase the formation of ROS, including nickel and titanium ions, in dental alloys (26). It has also been reported that nickel can generate free radicals from diatomic molecules and superoxide anions through a two-step process (21). Therefore, it is important to consider the potential risks associated with the use of nickel and cobalt dental alloys and take steps to minimise exposure to these metals.

Increased superoxide radical production also occurred in the coffee control group at a concentration of 31.25 µg/ml (Fig. 1A). Increased production of superoxide radicals in the GRCBE control group can occur since even though coffee acts as an antioxidant, its exposure to cells is still interpreted as foreign; hence, cells respond with an increase in superoxide radicals as a defence against foreign objects (27). Whereas, in all GRCBE therapy groups, the presence of GRCBE in Ni^{2+} and Co^{2+}-exposed PBMCs gradually decreased radical superoxide levels compared with that without GRCBE (Fig. 1B and 1C). Based on these results, there is cooperation between GRCBE and Ni2+ and Co2+, which can decrease superoxide radical production induced by the reactivity of Ni^{2+} and Co^{2+}. It also shows that GRCBE can act as an antioxidant in reducing superoxide radical production due to exposure to Ni^{2+} and Co^{2+}.

The efficacy of GRCBE in reducing free radical superoxide can be attributed to its high content of chlorogenic acid and other bioactive compounds. Green coffee beans are rich in antioxidants, which help reduce free radicals (11). Chlorogenic acid has multiple health benefits, including antioxidant, anti-inflammatory, and antihypertensive effects (12, 14). A study comparing the antioxidant activity of green and roasted coffee bean extracts found that both coffee bean extracts exhibited potent free radical scavenging properties (11). Another study examined the topical anti-inflammatory effects of the methanol extract of green coffee beans and reported positive results (12). These results support previous research indicating that GRCBE may be effective in reducing free radical superoxide and associated oxidative stress.

In addition, GRCBE can reduce superoxide radical production because coffee has a high antioxidant content, including flavonoids, xanthine alkaloids, and polyphenols (28). The antioxidants in coffee work through five main pathways that prevent chain reaction by scavenging ROS, donating one H electron to an unstable molecule so that it becomes a stable molecule, inhibiting oxidative enzymes, such as xanthine oxidase, protein kinase C, and others so that the production of ROS decreases, preventing the occurrence of chelation, which is a transition metal bond such as Fe_2 and Cu with O_2 and H_2O_2, which can form very active hydroxyl radicals, and repair damage that occurs in cells such as damage to lipid membranes, proteins, and acids deoxyribonucleic (DNA) cells (29, 30).

Flavonoids are part of the polyphenol group; they also have good health effects by counteracting free radicals. Flavonoids are good reducing compounds that can inhibit many oxidative reactions (31). Flavonoids are directly capable of binding to ROS, inhibiting enzymes that play a role in producing superoxide anions and preventing the peroxidation process by reducing alkoxyl and peroxyl radicals (32).

The caffeine compounds in coffee are xanthine alkaloids and chlorogenic acids, including polyphenol compounds. According to Saud Shah (2021), antioxidant activity is influenced by hydroxyl groups (33). The testing of antioxidant activity in caffeine and chlorogenic acid showed that chlorogenic acid has a higher antioxidant activity than caffeine because chlorogenic acid has many hydroxyl groups. Both compounds have antimutagenic, anticancer, and antioxidant activities that work against ROS. According to Kumar Shashank (2013), in the presence of hydrogen groups, flavonoids and phenolic compounds have free radical scavenging activity and play a role in preventing the formation of new free radicals by breaking chain reactions and turning them into more stable products (34).

TAK 242 and BAY 11–7082 are inhibitors of κB kinase (IKK) whose pharmacological effects include anticancer, neuroprotective, and anti-inflammatory effects. In this study, BAY 11-7082 pharmacological target pathways were further characterized to determine how the compound simultaneously inhibits different responses. These compounds are small molecule inhibitors that help block the signaling of Toll Like receptor 4 (TLR4) (35-37). TLR4 is a protein that plays a role in our system by recognizing and responding to bacterial lipopolysaccharides (LPS) (35). By inhibiting TLR4-mediated signaling, drugs such as TAK 242 and BAY 11-7082 can reduce the release of cytokines and safeguard against LPS-induced decrease in glucose transport (35, 36). Researchers have explored the potential of TAK 242 as a rheumatoid arthritis medication (36). Additionally, BAY 11-7082 has been studied for its ability to increase PDL1 expression through TLR4, thus supporting evasion, in small cell lung cancer (38). It has also been used to inhibit NFκB activation and phosphorylation of $I\kappa$Bα (17). Studies have demonstrated that TAK 242 can effectively reduce neuroinflammation in the cortex of rat brains following stress exposure (37). The present study confirms TAK 242 and BAY 11–7082 as p38 MAPK (mitogen-activated protein kinase) which give strong suppression of both nickel and cobalt (Fig. 4). This result is also in line with the previous experiment, which showed that the innate triggering capacity of nickel and cobalt is likely to contribute to its irritant properties and sensitizing capacity (17, 39). The inhibitory ability of the two blockers was then compared with the ability of GRCBE, and the results showed that the GRCBE concentration of 125 μg/ml had a significant ability to resemble the ability, especially to sensitivity to nickel and cobalt in reducing superoxide radicals.

Superoxide free radicals have both positive and negative effects on cells. Superoxide free radicals can serve as a cellular defense mechanism against foreign substances like Ni^{2+} and Co^{2+} ions, as well as provide protection against oxidative stress. (27). In contrast, superoxide radicals have the ability to induce oxidative harm to crucial cellular macromolecules, including proteins, lipids, and DNA, resulting in cellular damage and demise (40). Superoxide radicals can also promote the formation of hydroxyl radicals and the resulting DNA damage in all cell types (41). Excessive free radicals, including superoxide radicals, can cause cell damage and apoptosis, leading to many diseases and aging-related symptoms, such as wrinkles, cataracts, and age-related vision loss (42). Therefore, it is important for cells to maintain a balance between the production and scavenging of superoxide radicals to prevent oxidative damage and maintain cellular homeostasis.

It should be emphasized that the efficacy of GRCBE can differ based on variables such as extraction technique, concentration, and individual reaction. Additional investigation is required to comprehensively comprehend the possible advantages of GRCBE in diminishing free radical superoxide and its influence on overall health.

CONCLUSION

GRCBE can effectively reduce superoxide radical production in PBMCs exposed to $NiCl_2$ and $CoCl_2$. The optimal effective concentration for reducing superoxide radical production in this study was 125 μg/ml.

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